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# The Occurrence and Pathology of Dee Disease

By ISABEL W. SMITH, B.Sc., Ph.D.

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# The Occurrence and Pathology of Dee Disease

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EDINBURGH: HER MAJESTY'S STATIONERY OFFICE
1964

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# The Occurrence and Pathology of Dee Disease

## INTRODUCTION

The disease of salmon now known as Dee disease was first recorded in the Second Interim Report of the Furunculosis Committee (1933), where it is stated that during the spring and early summer of 1930, 1931 and 1932 fourteen salmon from the Aberdeenshire Dee and one from the River Spey were all found to have small necrotic lesions on their spleen. From these lesions a small Gram positive bacillus could be demonstrated but could not be grown successfully on culture media. Injections of splenic emulsions into brown trout proved lethal in three to five weeks so it was inferred that this organism was the causal organism of the disease. Since that time a number of cases of the disease have been reported. This paper gives an account of the occurrence of the disease and its pathology and compares it with kidney disease of salmonids reported by Earp, Ellis and Ordal (1953) and others in North America.

## OCCURRENCE OF THE DISEASE

As stated above this disease was first described in salmon from the Aberdeenshire Dee and the River Spey. Since then it has been reported in the Carron Water, Lower Inver and possibly in the River Beauly and Loch Assynt. Table 1 shows the source and number of fish examined each year. When the 18 examined during the period 1933-35 are discarded, as their source is unknown, it is found that of the 131 fish examined, all but 10 came from the Aberdeenshire Dee. This finding must be interpreted with care, as the number of dead fish received for examination may depend on the interest of the water bailiffs and on the ease of access to the laboratory, e.g. 81% of all the fish submitted for examination have come from the Aberdeenshire Dee.

Table 1 also shows that this disease is one which occurs in the spring and early summer (February to July). In the case of the Aberdeenshire Dee the majority of the fish are found in April, May and early June.

The number of fish examined in one year is never very large and only once in the past ten years has an epizootic been reported. This was in 1955 when there was a heavy snowfall in January, February and March which were the coldest on average in the 1954-61 period. The melting snows kept the river temperature low. On 24th May the water temperature at Invercannie (22 miles from the estuary) was 51°F and it rose steadily in almost daily intervals of one degree till it reached 61°F on 3rd June. On 1st and 2nd June 200 fish were reported dead at Park Bridge (14 miles from the estuary). Of these, 14 were submitted for examination and 12 were found to be suffering from Dee disease. The internal organs of the remaining two had been eaten out by eels and so were unfit for examination. In the other years only a small number of dead fish were found but here again there appeared to be some correlation between a rising temperature from 48° to 60°F and the incidence of Dee disease.

It is impossible accurately to assess the proportion of the fish in the Aberdeenshire Dee which are infected with Dee disease but in 1958, when a reward was offered from March to July for dead fish from lower Deeside, 52 specimens were received. Of these, 33 were suffering from Dee disease, 3 from furunculosis and 15 had died from no known cause. Thus it would appear that of the reported mortality in 1958 in the lower reaches of the Aberdeenshire Dee two-thirds was due to Dee disease.

## MACROSCOPIC SYMPTOMS OF THE DISEASE

### Salmon

There seem to be no diagnostic external symptoms of Dee disease in salmon, but fish suffering from this disease may show congestion of the fins, haemorrhage at the vent and, in a very few cases, haemorrhagic spots on the muscle. Such symptoms are found in other fish diseases so they are of little value

in the diagnosis of Dee disease. Internally the salmon shows very characteristic lesions, the most prevalent one being petechial haemorrhages of the muscle lining the peritoneum. These may be few in number but often they are very common (Plate 1A). The next organ most often affected is the kidney which, instead of its normal uniform reddish-brown colour, develops greyish-brown areas and, if the disease is more advanced, these areas develop an almost cyst-like appearance (cf. Bell, 1961). The spleen is the next in order. On its surface a number of creamish-white lesions, which vary in size from pin-head to about 4 mm. in diameter, develop. The number of such lesions also varies widely from one fish to another, ranging from a very few to a large number, which gives the spleen a peppered appearance. Such lesions can be hard and almost caseous but normally they are soft. Microscopical examination of smears from these lesions reveals a large number of Gram positive bacilli. These lesions, which are necrotic, are not confined to the surface of the organ but are found throughout its depth. The liver exhibits similar pathological changes (Plate 1B) but here the number of lesions is often smaller, and as a whole the liver is less often affected in this manner. Occasionally the swim bladder is covered with petechial haemorrhages in the same manner as the peritoneal muscle.

The symptoms just described are those most often found in Dee disease, but early in the year the pathology of this disease is somewhat different. At this time the spleen, liver, the gonads and even the swim bladder and peritoneal muscle are partially or completely covered with a white membrane (Plate 1C). This membrane is not firmly attached to any of these organs but can be peeled off leaving the surface of the organ apparently unharmed. In this type of infection, the pericardial sac is occasionally filled with a milky fluid consisting of tissue cells and the Gram positive bacilli.

There is no sharp boundary between the two forms and so in some salmon both the necrotic lesions and the white membrane are manifested (Plate 1D); here the membrane has been pulled back to reveal the apparently unharmed surface of the spleen.

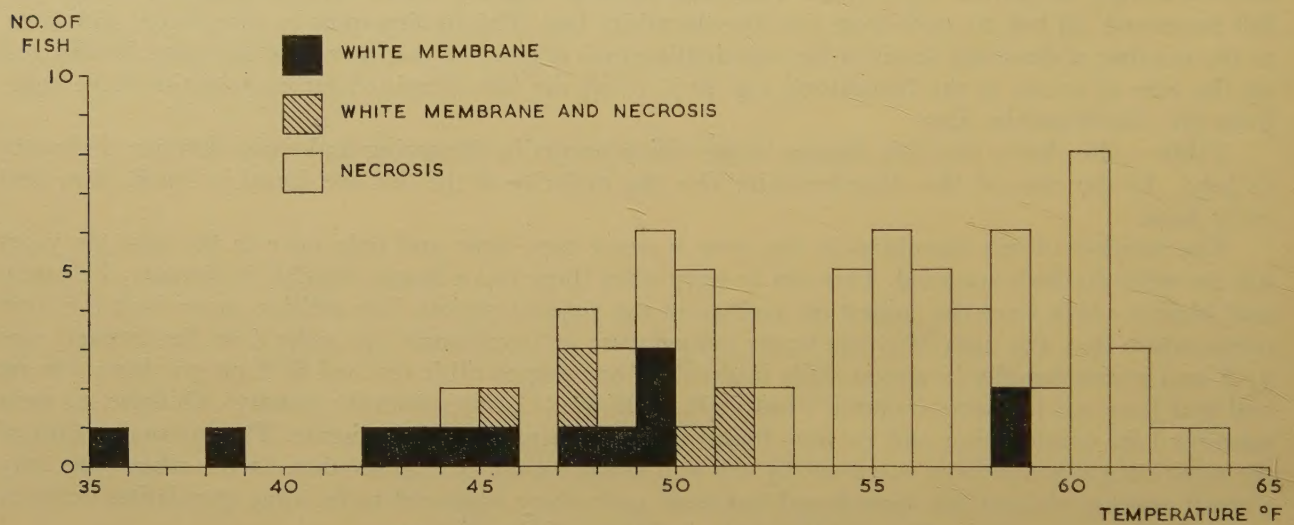



Fig. 1. The relationship between water temperature and the symptoms developed by salmon suffering from Dee disease.

The second clinical picture was thought to represent a chronic infection so the water temperature of the river was studied to see if there was any correlation of type of symptom with temperature. The results are shown in Fig. 1, in which the numbers of fish showing the different types of symptom are plotted against water temperature at Invercannie, which is within the section of the river (from the estuary to about 30 miles upstream) where dead fish were found. This figure shows that salmon which had white membranes on their spleen or liver were nearly all found dead when the water temperature ranged from 35° to 49°F; those with both white membranes and necrotic lesions from 45° to 51°F and necrotic lesions only from 44° to 63°F. Thus it may be said that at temperatures below 47°F salmon will most likely develop a white membrane whilst at temperatures above 49°F they will only show necrosis.

### Sea trout

In the past eight years only two sea trout have been shown to be infected with Dee disease. Both fish were from the Aberdeenshire Dee, one being a finnock and the other a kelt. In neither case was there any macroscopic symptom.

## MICROSCOPIC INVESTIGATIONS

In the routine investigation of specimens, Gram stained smears were prepared from the spleen, liver, heart blood, kidney and muscle. These showed the presence of the small Gram positive bacillus which is thought to be the causal organism of the disease. These bacilli are found both intra- and extra-cellularly, This microscopical examination was extended to include histological preparations of the spleen, liver and kidney. In both the spleen and the liver small areas of focal necrosis can be seen (Plate 2A). Around the edge of such an area the Gram positive bacilli are found while in the centre there is only tissue debris. In no case was giant cell-formation observed and in only a very few was there deposition of fibrin around these lesions. The kidney shows necrotic areas in a few cases but the organisms are much more often found in large numbers in the tissue surrounding the renal tubules.

In a number of sections of liver and spleen a 'false membrane' could be observed external to the capsule of these organs. The extent of this membrane varies from a very thin layer of fibrin and leucocytes which is not visible to the naked eye to a structure in which three layers can clearly be discerned (Plate 2C). In haematoxylin-eosin preparations the outermost layer appears to consist of fibrin and nucleated cells, the next of leucocytes showing nuclear fragmentation and being phagocytosed by histiocytes and the innermost one of fibroblasts and histiocytes. The Gram positive bacilli can clearly be seen in the outermost layer in Gram stained preparations while in Albert stained sections the bacteria are shown to contain metachromatic granules.

## ISOLATION OF THE PATHOGEN

The Second Interim Report of the Furunculosis Committee (1933) states that it had not proved possible to grow the pathogen successfully on ordinary culture media. Various media have therefore been tried in the past eight years but the organism was only isolated successfully when the blood-cysteine medium suggested by Ordal and Earp in 1956 for the cultivation of a similar organism was used. It was not possible to isolate the organism directly from all the specimens suffering from Dee disease because some specimens were contaminated with water organisms, eaten out by eels or infected with furunculosis in addition to Dee disease. As the Dee disease organism grows slowly, the cultures can be quickly overgrown with the faster growing contaminants or *B. salmonicida*. In 1958, when Ordal and Earp's medium was employed for the first time, the organism was isolated directly from 19 of 32 specimens. In 1959, when a modification of this medium was used (substitution of horse blood for human blood), the organism was isolated directly from the fish in only two cases out of 15. In 1960 the original recipe was used successfully in two cases but in 1961 the organism was not isolated from any of the four cases investigated. In addition to these direct isolates, three further strains were recovered from three trout which had been injected respectively with a suspension of the organs from two infected salmon and a finnock. These three fish were all too heavily contaminated to be useful for direct isolations but passage through the trout removed the contaminants.

Recently, Wolf and Dunbar (1959a) have suggested the use of Mueller Hinton agar with the addition of 0·1% cysteine for the cultivation of the causal organism of kidney disease. This agar was used for Dee disease but with very little success. Thus it appears that a medium really suitable for the isolation of the pathogen has still to be found.

A total of 26 strains was isolated from 1 finnock and 58 salmon of which at least seven were either eaten out by eels or additionally infected with furunculosis.

## DESCRIPTION OF THE ORGANISM

### Morphology

On Ordal and Earp's agar after 14 days at 15°C the cells were short rods measuring $1{\cdot}5\mu \times 0{\cdot}6\text{-}1{\cdot}0\mu$. They are non-motile, Gram positive, non-capsulate, non-acid-fast and non-sporing but sometimes contain metachromatic granules. There is no evidence of branching in young cultures but there is a little evidence of 'Chinese letter' formation.

### Colonial Appearance

*On Ordal and Earp's agar* the colonies are white, round, raised and shiny and of varying size. After prolonged incubation the growth becomes creamish-yellow but the colonies are still variable in size. The smaller ones tend to give the surface of the media a ground glass appearance. The growth has an elastic consistency.

*On Mueller Hinton agar plus 0·1% cysteine* the growth is very scanty. It is white, shiny, translucent and watery in consistency. There is not much evidence of colony formation.

*On Loeffler's coagulated serum* the organism produces a creamish growth with a matt surface.

*On Dorset's egg medium* the growth in 3-4 weeks at 15°C appears as a raised, smooth, shiny, yellow layer.

*On sea water egg medium* the growth is in the form of a raised, smooth, shiny and creamish yellow layer.

*On Ordal and Earp's medium without agar i.e. broth,* the growth is found adhering to the walls of the container at the surface of the broth. It is creamish-yellow in colour.

### Metabolic Properties

The organism grows very slowly at 5°C, well at 15°C, only very slightly at 22°C and not at all at 37°C.

### Biochemical Reactions

The organism does not grow on ordinary peptone water sugar media, Hiss's serum water sugars or semi-solid sugar agar containing Ordal and Earp base without blood. The use of the filter paper plaque technique on Ordal and Earp's medium produced no results so that nothing has yet been discovered about the enzyme system of this organism.

When *gelatin* is substituted for agar in Ordal and Earp's medium the organism grows reasonably well especially around the top surface of the medium but even prolonged incubation of up to 6 months does not show any liquefaction of the gelatin.

In *litmus milk* which has been made up with 25% Ordal and Earp's basal broth i.e. without blood or agar, the organism grows and it slowly produces proteolysis without any apparent pH change.

On Mueller Hinton agar catalase production can be demonstrated. This medium, unlike blood agar, does not contain catalase.

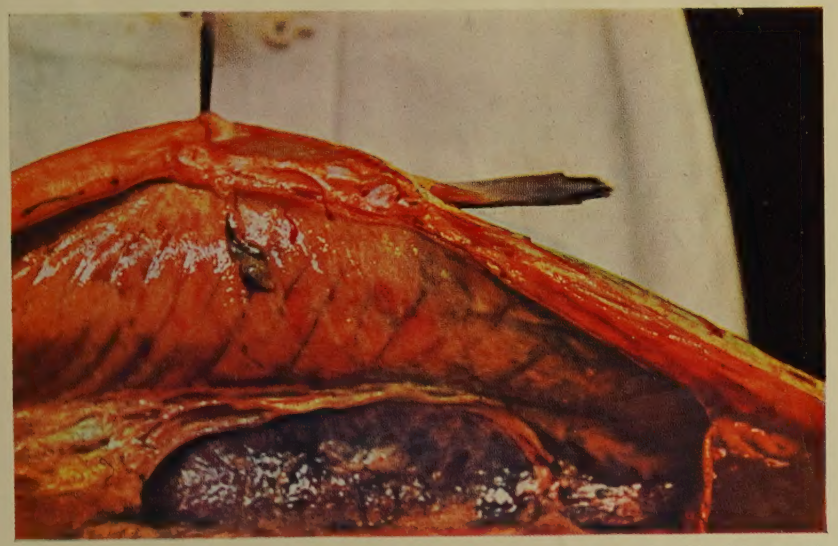
## PATHOGENICITY

As neither the sea trout kelt nor the finnock which was found to be naturally infected with Dee disease showed any macroscopic lesions, it was not surprising that very few of the sea or brown trout* or, for that matter, salmon parr inoculated experimentally, developed macroscopic lesions, although the organism could be demonstrated in smears from the organs of dead fish. This meant that neither trout nor salmon parr was the ideal experimental animal but both were used on account of convenience and it was concluded that a fish had died from Dee disease if the organism could be found in smears from the internal organs. No control trout or salmon parr ever yielded the Dee disease organism. (While using trout for experimental purposes, it was noted that quite a number were infested with *Ichthyophonus* spp. which manifested itself as white areas on the liver thus giving rise to some confusion with the pathological symptoms of Dee disease).

*Sea or brown trout are termed trout subsequently.

A. Muscle showing petechial haemorrhages.

B. Liver showing necrotic lesions.

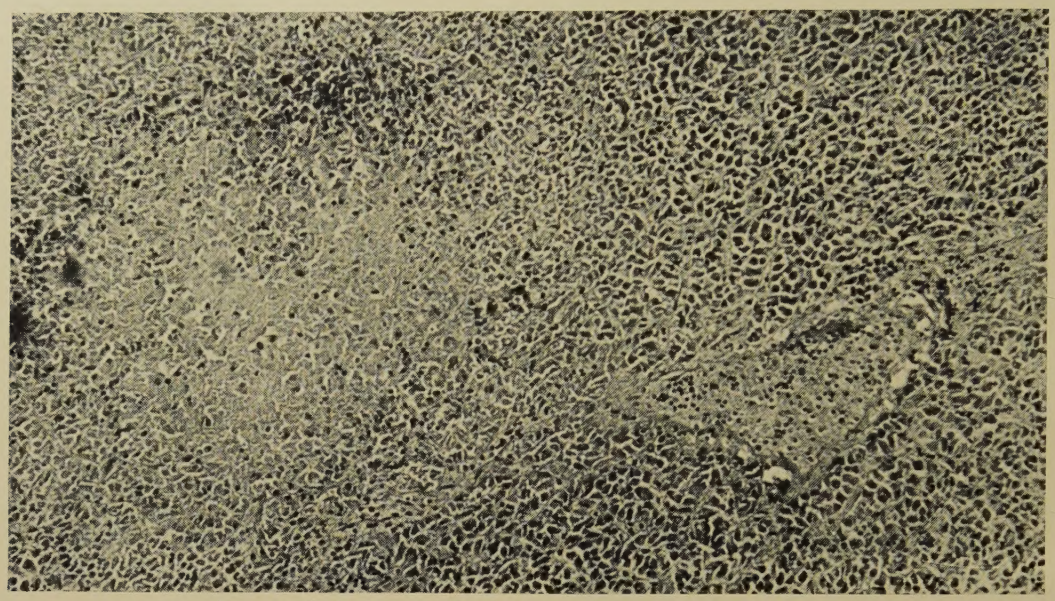


C. Membrane formation on the peritoneal muscle, swim bladder and kidney.

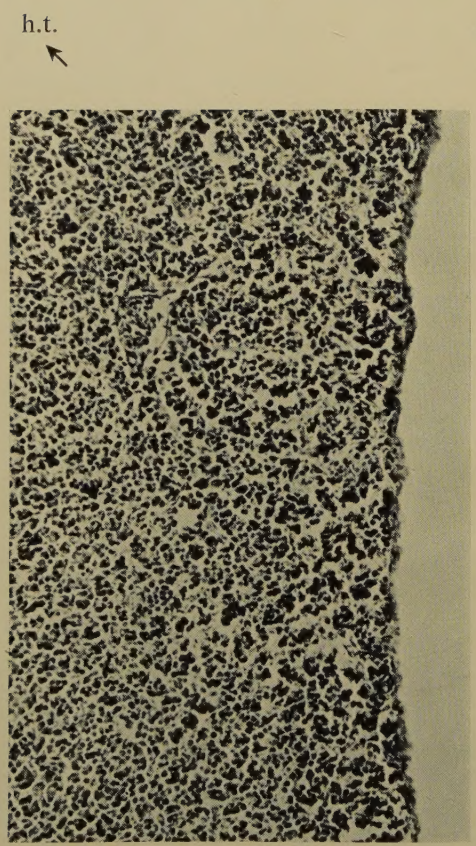
D. Membrane formation and necrotic lesions in the spleen.

PLATE 2

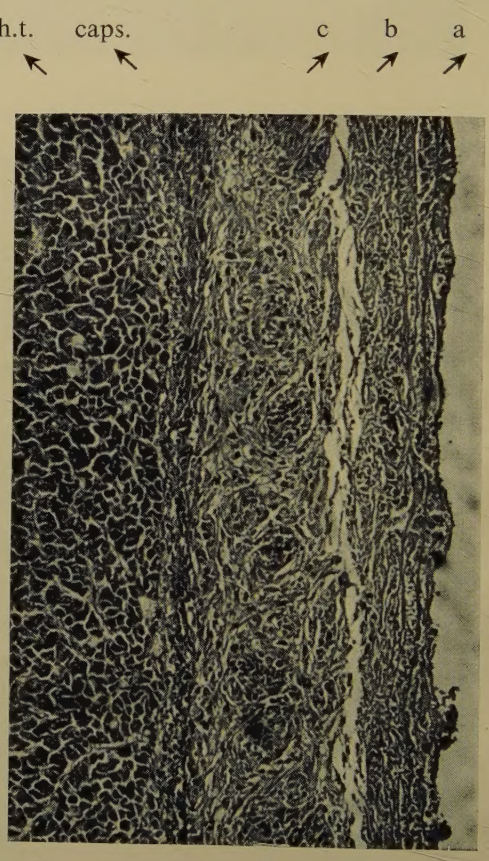
The microscopic appearance of Dee disease in sections of liver, X100 (stained haematoxylin and eosin).



A. A necrotic lesion on the left. On the right is a blood vessel.




B.




C.

B. Normal liver, showing the capsule (caps.) and uninfected hepatic tissue (h.t.).

C. Liver and membrane, showing the three layers of the latter. a. fibrin, uncleated cells and bacteria; b. leucocytes showing nuclear fragmentation and phagocytosis by histiocytes; c. fibroblasts and histiocytes; caps. capsule; h.t. hepatic tissue.

The trout and salmon parr were used for two main purposes.

(i) *As a means of isolating the organism from contaminated specimens.* The infected organs were removed from all the salmon and sea trout and stored in universal containers at $-17°C$. If the specimens were found to be too contaminated for direct isolation of the organism, saline suspensions were prepared from the deep frozen organs, checked microscopically for the presence of the Dee disease organism and injected intraperitoneally into trout or salmon parr. Table 3 shows that of 157 fish thus inoculated 40 died from Dee disease and 11 others from a combined infection of furunculosis and Dee disease. The causal organism was not re-isolated in every case as the early experiments were performed before any reliable medium for the cultivation of the organism had been devised and, even when a suitable medium was available, the experimental animals were often too contaminated due to their dying overnight. In this way, however, three strains of the organism which could not be directly isolated were recovered.

The large number of unsuccessful inoculations led to an appraisal of the condition and length of storage when it was noted that if organs were stored more than 14-16 weeks at $-17°C$, the small Gram positive bacilli, which were still present, were no longer able to cause the death of trout. This is in agreement with Ordal and Earp (1956) who state that organisms retain their virulence for up to 4 months when infected organs are stored in the deep freeze.

Saline suspensions of infected organs were passed through a Seitz filter (SB grade) to remove bacteria. Inoculations with such suspensions did not produce the disease whereas unfiltered suspensions from the same organs did. It was therefore concluded that the causal organism of Dee disease was a bacterium and not a virus.

(ii) *To test the virulence of the isolated organisms.* Nineteen of the strains of the organism which had been isolated were introduced intraperitoneally into trout or salmon parr and all such strains were shown to be virulent. Table 2 shows that of the 85 fish inoculated, 14 died from fungus, injury or jumping out of the tank, leaving 80% of the fish infected with Dee disease. These results have been based on smears from the liver and kidneys, but attempts were made to re-isolate the organism from the dead fish or to demonstrate it in liver sections. The cultivation of the organism was not always successful as the fish sometimes died overnight and so were contaminated with water organisms. However, the organism was re-isolated in the case of 15 strains and in two others the organisms were demonstrated in the liver sections.

Although there is a great deal of evidence above that the organism isolated from Dee-diseased salmon and sea trout is the pathogen, Koch's postulates had not been strictly fulfilled as a salmon had not been inoculated. Several salmon were obtained from time to time for this purpose but they almost without exception died from furunculosis. However, in 1959 a salmon from a river where the disease is not known to occur was inoculated with a culture of the organism and 50 days later it died. On autopsy white membrane formation was observed on its spleen and liver and the organisms were found in smears from all the organs. Subsequently the organism was re-isolated in culture, thus fulfilling Koch's postulates and establishing the small Gram positive bacillus as the causal organism of Dee disease.

The incubation period of this disease is difficult to establish as it seems to depend on a number of factors such as the virulence of the culture used, the resistance of the fish and the water temperature of the aquarium. There does not seem to be any direct relationship between the incubation time and water temperature so all that can be said at the moment is that (with pure cultures) the fish died in 5 to 62 days after inoculation and the average time was 22 days.

Very little has yet been done on the spread of the disease but to date there has been no evidence of its passing from one fish to another through water.

## DISCUSSION

Dee disease of *Salmo salar* is very similar to kidney disease which has been described in *Oncorhynchus tshawytscha, O. kisutch* and *O. nerka* by Earp, Ellis and Ordal (1953), in *Salvelinus fontinalis, Salmo trutta* and *Salmo gairdnerii* by Snieszko and Griffin (1955), in *O. tshawytscha* by Wood and Wallis (1955) and in *O. gorbuscha* by Bell (1961). A summary of the pathological symptoms recorded by these workers together

with those recorded in this paper is given in the Appendix. It can be seen that the first two and the fourth papers deal with young fish whilst the third and the present papers deal with adults. Comparison shows that exophthalmus is a symptom shown by the younger fish (except *O. gorbuscha* Bell, 1961) as is the production of a peritoneal effusion when fish were held in fresh water. Bell (1961) does not report this in *O. gorbuscha* when kept in sea water. The only effusion which has been recorded in adult *Salmo salar* is in the pericardial sac where in a few cases a milky fluid, consisting of large numbers of the organism together with tissue cells was found.

In the juvenile fish and the adult *Salmo salar* the authors report that the kidneys either exhibit creamish white necrotic lesions or are greyish white in colour. The liver and spleen of both juvenile and adult can show necrotic lesions but this is not such a constant syndrome as the kidney lesions except in the case of the adult *Oncorhynchus tshawytscha* where the liver is the organ which most often shows pathological changes. The muscle of both adult and juvenile fish show varying numbers of petechiae (Bell (1961) omits any reference to the muscle). Likewise, *Oncorhynchus tshawytscha* adults differ from the other fish in their lack of production of a false membrane. From the macroscopic syndromes it would therefore appear that Dee disease of *Salmo salar* is very similar to if not identical with kidney disease of *Oncorhynchus* spp., *Salvelinus fontinalis*, *S. trutta* and *S. gairdnerii*.

When the microscopic findings of the two diseases are compared, this similarity receives further confirmation. Wood and Yasutake (1956), who studied kidney disease in yearling and fingerling brook trout, yearling coho salmon and yearling fall chinook salmon, found granulomatous lesions between the renal tubules which closely resemble the lesions found in adult *Salmo salar*. Also in all four species of fish granulomatous nodules are found in the liver and spleen.

None of the previous workers has described the histology of the false membrane found on the surface of the liver and spleen, presumably as it occurs less often in juvenile fish and also it will be smaller. Initially this false membrane in *Salmo salar* was thought to be similar to that produced by *Coryne diphtheriae* on the human larynx but Ogilvie (personal communication) states that the salmon membrane differs from the diphtheritic one in that it is superficial and does not penetrate the hepatic or splenic capsule. In addition the salmon membrane shows organisation in its deeper layers and this is not a feature of the diphtheritic membrane. This membrane formation is being studied further and it is hoped that a fuller description will be published elsewhere.

The Scottish results appear to show that the water temperature influences the type of lesions manifested in the salmon. At low temperatures the membranous type is prevalent whereas at higher temperatures the necrotic type manifests itself. The American workers record the occurrence of both types of symptom (Earp, Ellis and Ordal, 1953; Snieszko and Griffin, 1955; Bell, 1961) but unfortunately they do not give any indication of the temperature at which these types occur or if they can actually be separated into two distinct forms.

The death rate at various temperatures has been compared by the American workers both in the field and under experimental conditions. Under experimental conditions Wolf and Dunbar (1959b) found that both the eastern and western strains of the organism were more virulent at 44·6°F than at 54·5°F when tested in eastern brook trout (*Salvelinus fontinalis*). Under field conditions Earp, Ellis and Ordal (1953) noted that most of the outbreaks of kidney disease produced a slow steady death rate in the declining water temperatures of winter. Similarly in the Aberdeenshire Dee there is a low mortality in the spring when temperatures are low. The only explosive epizootics reported by Earp, Ellis and Ordal (1953) were found in hatcheries where the water was warm and the only epizootic reported in Scotland happened when the water temperature rose rapidly early in the summer.

The extent of the disease among adult chinook salmon was found by Wood and Wallis (1955) to be 4·3% of 4287 fish examined prior to and following spawning (4·0% of the males and 4·9% of the females were infected). In Scotland it is impossible to estimate the extent of the disease but when the sex of the diseased fish is considered it is found that the majority of the fish are female (77 female : 36 male). This of course may be connected with the fact that the salmon are of a different genus.

The etiological organism of Dee disease is similar to that of kidney disease in that it is a small Gram positive diplo bacillus. Ordal and Earp (1956) give the size of the organism as 0·3-0·5$\mu$ × 0·5-1$\mu$ (pre-

sumably for stained organisms) and Bell (1961) quotes 0·5-0·7$\mu$ × 0·8-1$\mu$ for Gram stained organisms. The organism of Dee disease is 0·6-0·9$\mu$ × 1·0-2·8$\mu$ with an average length about 1·5$\mu$ which is slightly larger. This difference is due to the fact that it was done by wet mount. Ordal and Earp (1956) state that the organism is non-sporing, proteolytic and a catalase producer. The Scottish strain is also non-sporing, and a catalase producer but its proteolytic properties are very weak, it does not liquify gelatin even after long incubation and only very slowly proteolyses litmus milk.

When the little information available is used to classify the organism by Skerman's key for the determination of the generic position of organisms listed in the Manual (Bergey) it is found to belong to either the *Brevibacteria* spp. or the *Corynebacteria* spp. as it is a small, Gram positive, aerobic bacillus which does not produce endospores; is non-acid fast; does not branch; reproduces by binary fission and produces metachromatic granules. Skerman (p. 1104) states that the separation of these two genera is inadequate but the organism described here seems more closely related to the *Corynebacteria* spp. as it produces metachromatic granules, shows Chinese letter formation and is a pathogen. This is an agreement with the conclusions of Ordal and Earp (1956) and is further evidence of the similarity of kidney disease and Dee disease. The organism has now been compared with the American strains and found to be identical.

Little is known about the epizootology of kidney disease. American workers have tried to induce the disease orally but the success of this method appears to depend on the strain of the organism and the host fish used. Snieszko and Griffin (1955) could not transmit the eastern strain to brook trout orally whereas, using the western strain Earp, Ellis and Ordal (1953) claim success with the oral method in blue back fingerlings and Wood and Wallis (1955) in chinook fingerlings, although Earp, Ellis and Ordal (1953) had some kidney disease in their control fish. Wolf and Dunbar (1959b) again tried to infect brook trout orally and by abrasion with both the eastern and western strains. The oral route proved singularly unsuccessful whereas in the case of the eastern strain some deaths did occur after abrasion. This would appear to show that abrasion was a more important transmission route than feeding. The abrasive method is difficult to reconcile with Dee disease in adult salmon where there has been no great occurrence of noticeable wounds. Allison (1961) studied the effect of releasing brook trout from a stock infected with kidney disease into streams and lakes. He found no indication of the spread of the disease from the hatchery fish to the previous inhabitants.

Until we know something of the spread of this disease it will be difficult to combat it. In Northern America sulpha drugs (Rucker *et al.*, 1951) and erythromycin (Wolf and Dunbar, 1959a) have been employed in hatcheries with some success but it has to be long term treatment and could not of course be applied to Scottish rivers under field conditions.

## ACKNOWLEDGEMENTS

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## SUMMARY

1. Dee disease has been found mainly in the Aberdeenshire Dee and a few northern Scottish rivers in the spring and early summer.

2. The disease can have one of two forms. One is characterised by the production of necrotic lesions in the spleen and liver while in the other these organs are covered by a white false membrane. In both forms the muscle exhibits petechial haemorrhage.

3. The necrotic forms seems to occur from 44°-63°F and the membranous form from 39°-49°F.

4. The histology of the necrotic form shows it to be due to a granuloma whereas the membranous form is an inflammatory response involving the deposition of fibrin, tissue cells and the Gram positive rod.

5. The causal organism is a small Gram positive rod which can be grown on Ordal and Earp's blood cysteine agar, inoculated into a salmon where the characteristic lesions were produced and the organism re-isolated.

6. The organism most probably belongs to the *Corynebacteria* spp.

7. It is concluded that Dee disease is very similar to or identical with kidney disease.

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TABLE 1

The source and number of fish suffering from Dee disease

| Year | Source | Number | Time of Year |
|---|---|---|---|
| 1930-32 | Aberdeenshire Dee | 14 | Spring and early summer |
| | Spey | 1 | Spring and early summer |
| 1933-35 | | 18 | |
| 1938 | Beauly (?) | 1 | July |
| 1946 | Dee | 1 | May |
| 1947 | Spey | 1 | June |
| 1949 | Spey | 1 | June |
| | Lairg (Loch Assynt ?) | 1 | July |
| 1950 | Spey | 2 | May |
| 1951 | Carron Water | 1 | May |
| 1952 | Dee | 2 | April, May |
| 1954 | Dee | 5 | May |
| | Lower Inver | 2 | July |
| 1955 | Dee | 23 | April-June |
| 1956 | Dee | 3 | April-May |
| 1957 | Dee | 13 | April-May |
| 1958 | Dee | 33 | February-July |
| 1959 | Dee | 15 | April- July |
| 1960 | Dee | 8 | March-May |
| 1961 | Dee | 4 | April-June |

TABLE 2

Results of inoculation of trout and salmon parr with cultures of the isolated organism

| No. of trout and salmon parr inoculated | No. of fish which died from Dee disease | No. of fish which died from furunculosis, fungus, mechanical injury or which jumped out | No. of fish which did not succumb to any known cause | No. of strains inoculated | No. of strains producing Dee disease |
|---|---|---|---|---|---|
| 85 | 55 | 14 | 16 | 19 | 19 |

TABLE 3

Results of inoculation of trout and salmon parr with saline suspensions of organs from infected fish

| No. of trout and salmon parr inoculated | No. of fish which died from Dee disease | No. of fish which died from fungus, furunculosis, mechanical injury or jumped out | No. avirulent or negative | No. of strains inoculated | No. producing Dee disease |
|---|---|---|---|---|---|
| 157 | 40+11 with furunculosis | 25 | 75 | 38 | 21 |

## APPENDIX

### Summary of Pathological Symptoms Recorded

| Species | *Oncorhynchus tshawytscha*, *O. kisutch*, *O. nerka* | *Salvelinus fontinalis* *Salmo trutta* *Salmo gairdnerii* | *Oncorhynchus tshawytscha* | *Oncorhynchus gorbuscha* | *Salmo salar* |
|---|---|---|---|---|---|
| Age of fish | Fed 120-180 days | Hatchery stock of varying ages | Adult | Sept. of year of hatch. March of following year. | Adult |
| External symptoms | Sometimes exophthalmus | Distended abdomens, exophthalmus, blisters | No abdominal swelling or exophthalmus. Occasionally blisters in region of lateral line and abscesses in musculature | Swelling on caudal peduncle; small blisters on skin (these symptoms not always present) | No abdominal swelling or exophthalmus |
| Kidney | Most often affected. Dark colour, swollen, pasty consistency. Shows lesions which are watery pustules and, which when present in large numbers, give the kidney a greyish appearance | Swollen, greyish-white necrotic areas | Least affected organ | Greyish white lesions and necrotic areas. Most often affected | Greyish white. Second most common symptom |
| Liver and spleen | Less often affected. Lesions as kidney. May be covered by white membrane | Necrotic areas. Sometimes opaque, false membrane present | White water pustules; caseous necrotic areas or occasionally abscesses. Liver most often affected organ. Spleen less often | Greyish white lesions and necrotic areas. Spleen may have a translucent false membrane | Creamish white necrotic areas; white false membrane formation. Spleen more often affected than liver |
| Peritoneum | Light yellow, opaque, effusion | Light amber, semi-transparent fluid | No effusion | No increase in fluid | No effusion |
| Muscle | Petechial haemorrhages | Abscesses filled with nearly colourless or turbid fluid. Petechiae | Petechiae | | Petechiae— most common symptom |
| Authors | Earp, Ellis, and Ordal (1953) | Snieszko and Griffin (1955) | Wood and Wallis (1955) | Bell (1961) | Smith (1964) |

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